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Environmental Risk Factors

Principal Investigator: ANDERSON, MARJORIE
Grant Number: 5R01NS044565-03
Title: Deep Brain Stimulation in Parkinson's Models

Abstract: Although high-frequency deep brain stimulation (HF-DBS) in the globus pallidus or subthalamic nucleus has become a common technique used to treat drug-resistant symptoms of Parkinson's disease, the mechanisms by which HF-DBS exerts its effects are unknown. In the proposed studies, the ability of chronic administration of the insecticide rotenone, to produce an animal model of Parkinson's disease will first be tested in monkeys. Using PET imaging now available in the University of Washington Regional Primate Research Center, changes in dopamine innervation after administration of rotenone will be measured using a marker of the monoamine vescicular transporter that is present in dopaminergic nerve terminals. These changes will then be correlated, over time, with changes in behavior and with electrophysiological changes in the rate and pattern of discharge of neurons in basal ganglia-receiving areas of the thalamus. This model will then be used to couple the electrophysiological effects of HF-DBS, which can be recorded from basal ganglia-receiving neurons of the thalamus, to the stimulation-induced changes in regional metabolism in the cortex and thalamus. PET imaging with the metabolic marker, [8-F] flurodeoxyglucose (FDG), will be used to measure metabolism. This technique has generally shown a relative hypermetabolism in the globus pallidus and thalamus of humans with Parkinson's disease and a relative hypometabolism in areas of the frontal cortex. Changes reported to be induced by HF-DBS have been mixed however. The combination of electrophysiology and metabolic imaging will allow us to address some of the discrepancies from the human literature. Special attention will be paid to the development of abnormal patterns of bursting behavior in the thalamus of monkeys treated with rotenone, as well as the effect of HF-DBS on burst behavior. This will test the hypothesis that some of the symptomatology of Parkinson's disease, and its relief using HF-DBS, is a consequence of abnormal patterns of activity in basal ganglia-thalamic-cortical circuits.-

Principal Investigator: BENNETT, JAMES P
Grant Number: 5R01NS039005-05
Title: OXIDATIVE STRESS IN PARKINSON'S DISEASE

Abstract: Idiopathic Parkinson's Disease (PD) is a major neurodegenerative disease affecting at least 1 million Americans, and the cellular cause of PD is not yet known with certainty. This proposal will explore further the central hypothesis that defects in mitochondrial electron transport chain (ETC) function are a major contributor to premature cell death in PD and will address four Specific Aims 1) define the pathophysiology of mitochondrial transition pore function, and how regulation of membrane potential and intracellular calcium signaling are altered in PD; 2) determine mechanisms of Bcl protein regulation in PD cybrids, and whether transfection with Bcl-overexpression vectors alters mitochondrial function and improves survival; 3) further define the interactions among MAPKinase signaling pathways and NFkappaBeta transcription factor in PD; and 4) characterize mitochondrial transition pore complexes isolated from human postmortem PD brain and compare their function to those isolated from control brain. This project will make use of state-of-the- art intracellular ion imaging technology, RT-PCR techniques, gene transfection strategies, and will develop cell-free systems to examine several inter-related hypotheses. Behind all of these laboratory experiments is a therapeutic imperative, which will be explored in cell and cell-free models. Because new data presented in this application supports the hypothesis of systemically increased oxidative stress in PD patients, exploring these events in an established cell model is even more compelling. This proposal will also compare findings in PD cybrids with those in SY5Y cells exposed to chronic rotenone treatment, a pharmacological cell-based model of complex I loss. Ultimately, the results from this proposal will establish the central importance of genetically acquired mitochondrial ETC dysfunction as an etiologic factor in sporadic PD. Paradigms for evaluating neuroprotective therapies will also be developed to allow targeted approaches to correcting consequences of increased oxidative stress in cells. -

Principal Investigator: Berry, Marla J
Grant Number: 2R01NS040302-06
Title: Selenoprotein P function and regulation of expression

Abstract: There is a wealth of information implicating cumulative cellular injury inflicted by reactive oxygen species and heavy metal toxicity in neuronal damage and neurodegenerative diseases. Cells have evolved various endogenous antioxidant defenses to afford protection from oxidative injury or to reduce oxidative stress. Selenium is an essential contributor to these defenses, as it is required for the activity of a family of antioxidant enzymes that protect cells against the damaging products of normal oxygen metabolism. Selenium has also long been known to function as an antidote to toxicity of heavy metals. Selenoprotein P has recently been shown to function as a selenium delivery protein to brain, providing a source of this essential trace element for synthesis of other selenoproteins when selenium is deficient in the diet. Targeted disruption of the selenoprotein P gene results in neurological dysfunction. The overall goals of this study are to investigate the selenium delivery function of selenoprotein P in cells of neuronal origin, and to identify the crucial target selenoproteins which function in protection from oxidant and heavy metal induced damage, and which presumably explain the neurological effects of selenoprotein P gene disruption. These goals will be addressed through the following specific aims: 1. Investigate the means by which selenoprotein P serves as a Se donor to cells in culture, including interactions at the cell membrane and within the cell. 2. Investigate the expression levels and subcellular localization of specific selenoproteins, and whether localization or expression levels or patterns change in response to oxidative damage. Investigate the expression of specific selenoproteins in tissue sections from different brain regions in mice, and the changes in the expression levels or localization in response to GSH depletion or ischemia/reperfusion injury. 3. Identify the specific selenoproteins in neuronal cells responsible for protection from oxidative damage, resulting from either reactive oxygen species production or accumulation, or heavy metal induced damage. 4. Investigate expression of selenoproteins in brain sections obtained at autopsy from a cohort of Japanese men diagnosed with Alzheimer's or Parkinson's disease, vascular dementia, other neurological damage or with no evidence of neurodegenerative disease. -

Principal Investigator: BING, GUOYING
Grant Number: 5R01NS044157-02
Title: Cox-2 deficient mice are resistant to MPTP neurotoxicity

Abstract: Parkinson's disease (PD) is a movement disorder characterized by the progressive loss of dopamine-containing neurons in the substantia nigra pars compacta (SNpc). Loss of SNpc dopaminergic neurons results in the depletion of striatal dopamine levels and produces symptoms such as tremor, muscle rigidity, and bradykinesia. The etiology of PD is unknown, but chronic inflammatory processes, microglial activation, and oxidative stress are thought to play prominent roles in the degeneration of dopaminergic neurons in the SNpc. Microglia are thought to contribute to neurodegeneration by releasing cytotoxic agents such as pro-inflammatory cytokines and reactive oxygen species that increase inflammation and oxidative stress. N-Methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP) is a neurotoxin found to mimic many of the features of PD in animal models, including loss of dopaminergic neurons in SNpc and activation of microglia. Recent observations indicate that cyclooxygenase-2 (COX-2) deficiency in mice reduces the susceptibility of SNpc dopaminergic neurons to MPTP toxicity and diminishes MPTP-induced microglial activation. The purpose of this study is to test the hypothesis that COX-2-regulated inflammatory processes exacerbate MPTP neurotoxicity by activating microglia and increasing oxidative stress that contributes to the degeneration of dopaminergic neurons in the SNpc. To test this hypothesis, mice deficient in the COX-2 gene will be treated with MPTP to determine the role of COX-2 in MPTP-induced neurodegeneration. Furthermore, wild-type mice will be administered exogenous COX-2 inhibitors prior to MPTP treatment to evaluate the protective effects of COX-2 inhibitors against MPTP neurotoxicity. Following these experiments, dopaminergic neuron survival, microglial activation, striatal dopamine levels, and functional recovery will be assessed. In addition, protein modification, generation of reactive oxygen species, expression of inflammatory cytokines and apoptosis-related genes, and activation of specific signaling molecules will be evaluated to determine the molecular mechanisms by which COX-2 exacerbates MPTP neurotoxicity. The goals of this study are to elucidate the changes in inflammatory processing affected by COX-2 deficiency, to explore the etiology and molecular mechanisms underlying Parkinsonian symptoms in the experimental MPTP model, and to develop novel therapeutic treatments for PD and other neurodegenerative diseases.-

Principal Investigator: CHEN, HONGLEI
Grant Number: 1K08NS048468-01
Title: Diet, gene-diet interactions and risk of Parkinson's

Abstract: The candidate, Honglei Chen, M.D., Ph.D., has more than two years research experience in Parkinson's disease (PD) and is currently a Research Associate at Harvard School of Public Health. Dr. Chen's research interest includes the environmental and genetic etiology of sporadic PD and that of other neurodegenerative diseases, and he plans to develop an independent academic career in this area. In this K08 proposal, Dr. Chen proposes a large prospective investigation of diet and risk of sporadic PD in the Cancer Prevention Study-ll Nutrition Cohort (CPS-IIn) and a large nested case-control study of PD with genetic polymorphisms and gene-diet interactions in the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS). In the CPS-IIn, he will prospectively examine among 162,408 US men and women associations of PD with dietary intakes, focusing on folate, coffee, dietary antioxidants, fat, alcohol, and dairy products. Confirmation of incident PD cases in CPS-IIn is ongoing and they expect to document 550 definite and probable PD cases diagnosed between 1992 and 2001. In the HPFS and NHS cohorts, he will evaluate the associations of PD risk with common polymorphisms of NAT2, CYP1A2, ADH2, ADH3, ADH4, and MTHFR. He also will, for the first time, explore gene-diet interactions in PD etiology, including NAT2, CYP1A2 and caffeine intake; ADH2, ADH3, ADH4, and alcohol intake; and MTHFR and folate intake. Through the year of 2000, they have documented 567 definite and probable PD cases and 454 of them provided either blood or cheek cells for genetic analysis. In this proposed nested case-control study, two controls will be selected for each PD case matching on age and gender. All three cohorts included in this proposal are well-established large prospective cohorts with comprehensive (baseline and updated) and validated dietary assessments and rigorous outcome ascertainments. Moreover, the scope of this study makes it one of the largest investigations to date. The completed or nearly completed data collection will further make this study most cost-effective. Therefore, this K08 grant will simultaneously accomplish two important goals: helping Dr. Chen develop the skills to become an independent researcher in the epidemiology of neurological diseases and furthering our understanding of the complex interrelationships among diet, genes and PD etiology. -

Principal Investigator: DAWSON, TED M
Grant Number: 1R21NS047565-01
Title: Models of Familial Parkinson's Disease: DJ-1 Knockouts

Abstract: Mutations in the DJ-1 gene are a rare genetic cause of autosomal recessive Parkinson's disease (PD). The DJ-1 protein is either absent or appears to be functionally inactive in the families in which mutation have been identified. Thus, mutations in the DJ-1 gene probably cause PD through a loss of function. It is difficult at this juncture to fully appreciate how mutations in the DJ-1 gene cause PD, as its function is largely unknown. DJ-1 was identified as a hydroperoxide-responsive protein that becomes more acidic following oxidative stress suggesting that it may function as an antioxidant protein. Furthermore, DJ-1 is sumoylated through binding to the SUMO-1 ligase, PIAS, suggesting that it might be involved in the regulation of transcription. Other putative functions of DJ-1 have been raised, but how a loss of function of DJ-1 leads to loss of DA neurons and PD awaits further study. We propose to generate and characterize DJ-1 knockout mice to formally test the hypothesis that the absence of DJ-1 function is the cause of PD due to DJ-1 mutations. Accordingly experiments are proposed to further characterize the role of DJ-1 in the pathogenesis of PD. In Specific Aim #1 we will develop and characterize DJ-1 knockout mice. In Specific Aim #2 we will evaluate the sensitivity of DJ-1 knockouts to environmental toxins including MPTP-induced dopaminergic cell death. In Specific Aim #3 we will determine whether DJ-1 interacts with parkin by evaluating the effect of crossing DJ-1 knockout mice with parkin knockout mice. Development and characterization of DJ-1 knockouts, understanding the relationship of DJ-1 and parkin in the pathogenesis of PD may provide insight into the molecular mechanisms by which these gene products induce neuronal damage and may provide novel therapeutics and targets to prevent the toxic effects of these familial associated genes in the degenerative process of PD. -

Principal Investigator: DAWSON, TED M
Grant Number: 1R01NS048206-01
Title: The Role of Parkin in Parkinson's Disease

Abstract: Mutations in the parkin gene are the main genetic cause of autosomal recessive Parkinson's disease (PD) and mutations in parkin also play a major role in familial Parkinson's disease. Preliminary studies indicate a potential pivotal role for parkin in the ubiquitin proteasomal pathway (UPP) by functioning as an ubiquitin E3 ligase. Most disease causing mutations of parkin are thought to be loss of function mutations that ultimately lead to the absence of ubiquitination and the subsequent failure of UPP-mediated degradation of parkin substrates. Thus, the abnormal accumulation of parkin substrates is thought to play a role in the demise of substantia nigra dopaminergic neurons in patients with parkin mutations. A number of putative parkin substrates have been identified, but their importance in the pathogenesis of PD due to parkin mutations is not known. We propose to generate and characterize parkin knockout mice to formally test the hypothesis that the absence of parkin function is the cause of PD due to parkin mutations. Furthermore, biochemical and proteomic characterization of the parkin knockout mice may shed light on the substrates that are important in the pathogenesis of PD due to parkin mutations. Accordingly experiments are proposed to further characterize the role of parkin and it's substrates in the pathogenesis of Parkinson's disease. In Specific Aim #1 we will develop and characterize parkin knockout mice. In Specific Aim #2 we will evaluate the sensitivity of parkin knockouts to environmental toxins including MPTP-induced dopaminergic cell death. In Specific Aim #3 we will evaluate the interaction of parkin with the alpha-synuclein interacting protein, synphilin-1 and determine whether parkin mediates K48 or K63 ubiquitin linkages. In Specific Aim #4 we will determine whether parkin interacts with alpha-synuclein and evaluate the effect of crossing parkin knockout mice with A53T mutant alpha-synuclein transgenic mice. In Specific Aim #5 we will identify and characterize parkin interacting proteins in parkin knockout mice. Development and characterization of parkin knockout mice, understanding the relationship of parkin, alphasynuclein and synphilin-1 in the pathogenesis of PD may provide insight into the molecular mechanisms by which these gene products induce neuronal damage and may provide novel therapeutics and targets to prevent the toxic effects of this familial associated genes in the degenerative process of Parkinson's disease. -

Principal Investigator: DICKSON, DENNIS W
Grant Number: 2P50NS040256-06
Title: Genetics and Molecular Biology of Parkinsonism

Abstract: The Udall Center for Excellence in Parkinson's Disease Research at the Mayo Clinic is an integrated, multidisciplinary center that studies the Genetics and Molecular Biology of Parkinsonism. The Center draws upon the clinical strengths of the Mayo Clinic Movement Disorder Section as well as epidemiologic and longitudinal studies of Parkinson's disease (PD), dementia with Lewy bodies and aging that provide clinical material for research projects. The Clinical Core is a multi-national effort to identify and characterize multiplex families with PD for genetic studies of PD. The Clinical Core also recruits and follows sporadic PD patients and arranges for postmortem studies. The Genetic Core provides genetic screening and performs genome wide linkage studies of familial PD. When permission is granted, samples are submitted to the NINDS DNA repository. The Neuropathology Core performs postmortem evaluations of PD, provides histologic support for projects and provides postmortem material collected through several different avenues for the research projects. Project 1 builds upon progress from the previous funding period demonstrating multiplication of the alpha-synuclein gene (SCNA) in autosomal dominant, early-onset PD and focuses on population genetics of SNCA, characterization of SNCA multiplications (including the size and genes within the multiplication regions), and measuring temporal and regional alpha-synuclein expression in normals and a-synucleinopathies. Project 2 is a clinicopathologic study that determines the frequency and clinical expression of Lewy bodies in normal individuals using the Mayo Medical Records Linkage System, with studies on the role of neuronal loss, inflammation and tau on clinical features. Project 3 uses cell lines that inducibly express alpha-synuclein as well as mitochondrial toxins, such as rotenone, to study truncated and aggregated alpha-synuclein with the goal of determining the role of interacting proteins in aggregate formation and the effects of aggregates on proteasome function and gene expression.-

Principal Investigator: DIXON, C EDWARD
Grant Number: 3R01NS033150-09S1
Title: CHRONIC CHANGES IN NEUROTRANSMISSION FOLLOWING TBI

Abstract: Unavailable

Principal Investigator: EAKIN, CATHERINE M
Grant Number: 5F31NS046937-02
Title: Mechanisms of divalent cation associated amyloidosis

Abstract: The conversion of normally soluble proteins into amyloid fibers has pathological and functional consequences in a number of human diseases. A general cause for amyloid formation is not known. However, in many types of fiber formation, interaction of the protein precursors with divalent metals promotes aggregation. Divalent metals, particularly Cu2+, have been implicated as a central component in the formation of amyloid fibers in an increasing number of diseases. These include amyloid-beta in Alzheimer's, prion protein in Creutzfeldt-Jakob Disease, immunoglobulin light chain in Light Chain Amyloidosis, alpha-synuclein in Parkinson's, and beta-2-microglobulin (beta2m) in Dialysis Related Amyloidosis (DRA). Interaction with divalent metals may act to induce novel structure, preferentially bind amyloidogenic intermediates, or catalyze the sampling of a refolding pathway which contains amyloidogenic intermediates. The experiments proposed here will investigate the molecular basis for divalent metal associated amyloid formation in DRA. The first aim of this work is to determine the kinetics and pathway of Cu2+ associated fibrillogenesis of beta2m. The second aim is to determine the structure of a well-defined amyloidogenic precursor formed in the presence of Cu2+. The third aim is to determine the unfolded state structure of beta2m formed in the presence of divalent metal. These experiments will aid in understanding amyloid formation in DRA, but also contribute to establishing a general model for divalent metal associated amyloid formation.-

Principal Investigator: GILBERT, JOHN R
Grant Number: 5R01NS043473-03
Title: Genetics and Epidemiology of Essential Tremor

Abstract: Essential Tremor (ET) is a heterogenous tremor disorder characterized by a core group of features. The tremor syndrome is characterized by postural and kinetic tremor affecting the arms and hands, although the head, voice, and legs may also be affected. Although frequently described as a benign disorder, this is not true; many patients are socially and physically handicapped, with some patients being totally disabled. The differential diagnosis list for ET is extensive including dystonia, Parkinsonism, myoclonus, peripheral neuropathy, and other conditions. Prevalence estimates range widely, depending upon methodology and diagnostic criteria, from 0.003 to as high as 2% in the general population, with as much as 5% of the population affected over the age of 65. There are no known biological or diagnostic neuropathological markers for ET. The estimates of ET cases presenting with a positive family history range from 17.4% to 100%. Recent studies indicate that up to 96% of ET may be dominantly inherited. Clinical and genetic heterogeneity have slowed linkage studies. To date three loci associated with ET have been linked: 1) Familial Essential Tremor 1 (FET1) has been mapped in a series of Icelandic families on chromosome 3q13; (2) ETM mapped, in four unrelated US families, to chromosome 2p22-p25; and (3) a third locus maps, in a family that segregates both Parkinson's disease and postural tremor consistent with ET, to Chromosome 4p. We have, to date, ascertained, twelve ET and ET/PD linkage quality families. The largest pure ET kindred (DUK13001) have been excluded from known ET loci. The aims of this proposal are to ascertain and sample large families with ET, carry out a complete ET genome scan to establish linkage for these and additional ET families, identify new ET disease loci, and isolate and characterize ET genes, beginning with DUK13001 ET family.-

Principal Investigator: Goldstein, David
Grant Number: 5Z01NS002979-06
Title: Clinical Neurocardiology: Catecholamine Systems In Stress And Disease

Abstract: Unavailable

Principal Investigator: Higgins, Joseph J.
Grant Number: 5R01NS039353-05
Title: POSITIONAL CLONING OF A GENE FOR ESSENTIAL TREMOR

Abstract: Essential tremor (ET), the most common movement disorder in humans, significantly compromises the livelihood or social function of at least 85 percent of the 4 million individuals affected with the disease in the United States. Aggravated by emotions, hunger, fatigue and temperature extremes, the condition may cause a functional disability or even incapacitation. The main clinical feature of ET is postural tremor of the arms, but the head, legs, trunk, voice, jaw, and facial muscles also may be involved. The majority of cases are familial and the disease is usually an autosomal dominant trait with incomplete penetrance. The identification of two susceptibility loci on chromosomes (chr) 2p22-p25 (ETM) and chr 3q13.1 (FET1) implies that ET is genetically heterogeneous. We originally identified the ETM locus in a single American family of Czech descent with pure ET, and later refined the location of the ETM gene to 9.1 centiMorgan region by genotyping three additional families with a similar phenotype. The long-term objectives of the proposal are to identify the other ET susceptibility loci by linkage analysis and to characterize these genes by positional cloning techniques. The specific aims are the following: 1). Collect additional individuals and families with ET. 2). Define the minimal critical region (MCR) that contains ET genes by identifying key recombinants. 3). Construct a high-resolution physical map (contig) of the MCR. 4). Isolate the genes within the contig and evaluate these candidates for disease-causing mutations. The results of this research will enhance our understanding of the human motor system in general and the pathogenesis of tremor in particular. Because current pharmacological treatments for ET have limited efficacy and often become ineffective with advancing disease, identifying the genes that cause ET will facilitate the development of more effective therapeutic strategies. -

Principal Investigator: KALYANARAMAN,
Grant Number: 2R01NS039958-05
Title: Role of Neuronal NOS & Superoxide in Neurodegeneration

Abstract: Long-term goal: The broad objectives of this renewal are to understand the mechanism(s) by which mitochondrial neurotoxins such as 1-methyl-4-phenylpyridinium (MPP+) selectively destroy dopaminergic neurons in the substantia nigra, leading to the development of Parkinson's disease (PD). Reactive oxygen and nitrogen species (ROS/RNS)-mediated damage has been implicated in age-related neurodegenerative diseases like PD. Hypothesis: (i) MPP+ generates mitochondria superoxide (02*) and hydrogen peroxide (H202), and inactivates mitochondrial iron-sulfur-proteins (e.g., aconitase). This stimulates transferrin receptor (TfR)-mediated uptake of iron. (ii) MPP+-induced H202 and iron transported through TfR cause enhanced degradation of tetrahydrobiopterin (BH4), an essential co-factor for neuronal nitric oxide synthase (nNOS), tyrosine hydroxylase (TH), and dihydropteridine reductase (DHPR) activities. BH, depletion causes "uncoupling" of nNOS to form O2* and inactivation of TH and DHPR leading to dopamine depletion. (iii) MPP+-induced O2*, H2O2, and Tf-iron stimulate aggregation of a-synuclein, a neuronal presynaptic protein leading to apoptosis or programmed cell death. Aims: 1.) Investigate the effect of TfR-dependent iron and mitochondrial ROS in neuronal cell apoptosis in response to MPP+. 2.) Assess the modulatory effect of BH4 depletion on nNOS-generated nitric oxide ('NO)/O2* ratio and on BH4-dependent enzyme controlling dopamine synthesis. 3.) Elucidate the role of ROS, Tf-iron and BH4 depletion on a-synuclein aggregation and apoptosis in neuronal cells treated with MPP+. Methods: We will use both dopaminergic and non-dopaminergic cells (neuroblastoma and cerebellar granule neurons). The following redox-parameters will be measured: GSH and lipid peroxides; aconitase, complex-I, and iron-regulatory activities; TfR expression and 55Fe uptake; a-synuclein expression and aggregation; caspase activation and apoptosis. ROS/RNS will be determined by fluorescence and spin-trapping techniques. Significance: PD affects about 1% of population over the age of 50. Emerging data allude to environmental mitochondrial toxins as a causative factor. Novelty: This proposal sheds new light on the synergistic role for MPP*-induced mitochondrial ROS, iron, BH4-induced nNOS uncoupling, dopamine depletion and alpha-synuclein aggregation in neuronal toxicity of PD and other mitochondrial diseases.-

Principal Investigator: Kanthasamy, Anumantha
Grant Number: 5R01NS045133-02
Title: CASPASES, MITOCHONDRIAL FUNCTION AND PARKINSON'S DISEASE

Abstract: Parkinson's disease (PD) is a major neurodegenerative disorder affecting approximately 2% of the population over age 50, and the number of annual PD cases continues to rise along with the median age of the population. As the population in our society ages, we face the regrettable reality that effective medical treatment strategies for major chronic neurodegenerative disorders, including Parkinson's disease, are lacking. Determining the mechanisms of etiopathogenesis and selective nigrostriatal degeneration in PD is a formidable challenge. Emerging epidemiological and case control studies suggest that environmental factors, especially pesticides, are dominant risk factors in the etiology of sporadic, geriatric-onset Parkinson's disease. In this proposal, our preliminary data reveal that dopaminergic cells are susceptible to Dieldrin (a potential environmental risk factor for development of PD) -induced apoptosis, in which oxidative stress plays a causal role. We have also uncovered a novel apoptotic pathway involving caspase-3 dependent proteolytic cleavage of protein kinase Cdelta (PKCdelta) that not only mediates apoptosis in dopaminergic cells, but also influences key cellular events such as amplification of the apoptotic cascade through positive feedback activation and hyperphosphorylation of alpha-synuclein. We will extend our preliminary findings by pursuing the following Specific Aims: (I) characterize mitochondrial dysfunction and the subsequent activation sequence of key proapoptotic factors during dieldrin-induced oxidative stimulation in the mesencephalic dopaminergic cell model of Parkinson's disease, (ii) establish the proapoptotic function of caspase-3 dependent proteolytic activation of PKC5 in Dieldrin induced dopaminergic degeneration and to further investigate mechanisms underlying positive feedback amplification of the apoptotic signaling cascade by PKCdelta, (iii) obtain evidence to support the hypothesis that proteolytically activated PKCdelta hyperphosphorylates alpha-synuclein and thereby promotes protein aggregation, (iv) examine whether chronic exposure to Dieldrin in animal models induces caspase-3 dependent proteolytic cleavage of PKCdelta, alpha-synuclein aggregation, Lewy body formation and apoptotic cell death of dopaminergic neurons in the substantia nigra, and finally, (v) confirm the involvement of PKCdelta in nigral dopaminergic degeneration by using PKCdelta knockout animals and by targeted over-expression of PKCdelta and alpha-synuclein using a lentiviral delivery system in animal models. Together, results from the proposed systematic investigation will demonstrate the involvement of mitochondrial dysfunction, oxidative stress, apoptosis and

Principal Investigator: LAWRENCE, MATTHEW S
Grant Number: 1R43NS048786-01
Title: Genomic markers of environmental toxins for Parkinsonism

Abstract: Parkinson's disease is a prevalent and devastating neurodegenerative condition of unknown etiology. One prominent hypothesis holds that the selective loss of the nigrostriatal dopaminergic neurons characteristic of the disease results from damage from environmental neurotoxins in genetically vulnerable individuals. Identifying such environmental contributors to Parkinson s pathogenesis represents a significant public health concern. This project aims to identify the in vivo gene expression changes that occur in the primate brain in response to environmental toxins that have been implicated in the production of Parkinson's and compare these changes with the selective neurotoxin, MPTP, and with the limited knowledge of genetic abnormalities in some Parkinson's patients. Because of the unique vulnerabilities of nonhuman primates and humans to dopamine neurotoxic agents, studies in primates are essential to uncover common genetic markers of toxicity and to reveal the potential toxicity of chemicals of unknown liability. The proposed Phase I studies will test the hypotheses that transcriptional changes that accompany and precede dopamine cell death can be identified using high density gene arrays and bioinformatics in the primate nigrostriatal system in vivo following MPTP exposure. Changes in mRNA initiation of regimen of 3 doses of MPTP over 36 hours that has been established to result in Parkinsonism. Expression changes will also be assessed 6 hours after the administration of a single dose. Changes in nigrostriatal dopamine concentrations and tyrosine hydroxylase immunohistochemistry will be assessed at all time points. Additionally neurobehavioral changes will be assessed in the 20-day animals. Together these data will allow a determination of the sequence of transcriptional changes that parallel or precede histological, biochemical and behavioral events, and allow an assessment of transcriptional events related to acute versus chronic toxicity, with confirmation by quantitative RT-PCR. Defining the chronological and dose dependent gene expression changes induced by MPTP may reveal a transcriptional profile that is predictive of nigrostriatal injury from this toxin. Phase II studies will address whether similar gene expression changes and neuronal injury are seen following exposure to environmentally prevalent compounds that are postulated to be risk factors for the development of Parkinson's disease, and to integrate the resulting transcriptional data into a toxicogenornic database and potentially customized microarrays which may be applied to the assessment of compounds for their possible health risk.-

Principal Investigator: LEE, STEPHEN L
Grant Number: 5K08NS044298-02
Title: Genetic Analysis of Parkinsonism in an Ohio Amish Family

Abstract: Disease genes discovered through linkage analysis in familial Parkinson disease (PD) are yielding new insights into the pathogenesis of this neurodegenerative disorder. However, the known genes explain only a minor portion of all PD, and the chromosomal regions linked to other families are large and contain numerous genes. The discovery of additional hereditary causes of PD may help further elucidate the underlying etiopathogenesis and provide new pharmacological targets. It is therefore crucial that additional families are characterized. In an extended Amish family in northeastern Ohio, clinical information for familial Parkinsonism has been obtained. To test the hypothesis that genetic influences contribute to the expression of Parkinsonism in this Amish pedigree, the immediate aims of this project are three-fold: 1) to fully ascertain the disease phenotype of the affected individuals, through genealogical data, clinical history, medical records, and neurological exam, 2) to identify the genetic locus or loci associated with the disease phenotype, initially by evaluating previously identified genetic loci, and conducting a genome-wide scan using conventional linkage analysis, transcript mapping, and gene identification, and 3) to perform candidate gone analysis to test whether specific gene modifiers enhance or suppress the expression of the disease phenotype. The candidate's long-term goals are to apply well-established and emerging methods toward understanding the genetic basis of Parkinsonism. This grant will help the candidate establish an independent career in academic neurology with specialization in movement disorders and neurogenetics by allowing the candidate 1) to evaluate and treat patients in a movement disorders clinic under the guidance of Thomas L. Davis, M.D., and 2) to learn the laboratory, statistical, and computational methods of genetic epidemiology. This will be accomplished through conduct of the proposed research project and participation in formal courses under the guidance of the candidate's mentor, Jonathan L. Haines, Ph.D.-

Principal Investigator: MARDER, KAREN S
Grant Number: 2R01NS036630-05A1
Title: Genetic Epidemiology of Parkinson's Disease

Abstract: In the first funding period, we compared the risk of PD in relatives of 221 PD patients with age at onset (AAO) < 50 to relatives of 266 PD patients with AAO >50 and 409 controls. The magnitude of increased risk of relatives of PD cases vs. controls was similar in early-onset cases (RR: 2.9, 95%C1: 1.6-5.0) and late onset cases (RR: 2.7, 95%CI: 1.6-4.4). However in families of early-onset cases, the degree of increased risk was much greater in siblings (RR: 7.9, 95%C1: 2.5-25.5) than in parents (RR: 1.7, 95% CI: 0.9-3.3), consistent with an autosomal recessive contribution to inheritance. In late-onset families, risk was elevated in both parents and siblings, inconsistent with a recessive model. Mutations in the parkin gene have emerged as the most important causative or risk-raising factor in early-onset PD. In this second competitive renewal application, we have redesigned our study to make optimal use of 300 early onset cases already recruited at the Columbia site (200 not yet screened for parkin mutations) and the 40 PD cases with parkin mutations we have already identified. We will join with investigators at 7 other sites who will contribute an additional 600 cases with age of onset < 50 and 21 identified parkin families to form a US Parkin Consortium. Our first aim is the expansion of 125 PD cases that carry parkin mutations to include 1st and 2nd degree relatives. We will determine whether the risk of psychiatric and cognitive manifestations in asymptomatic gene carriers who do not meet criteria for PD is higher than in asymptomatic non-gene carriers. Identification of a parkin carrier phenotype will provide clues to etiopathogenesis and may define the appropriate time for early therapeutic intervention. Our second aim is to define the distribution of age specific penetrance in 100 of the 125 families who were recruited solely be age of onset, so as not to bias these estimates upward by inclusion of "high risk" families recruited because they are multiplex. We will compare differences in age specific penetrance by allelotype (heterozygous vs. homozygous or compound heterozygous). The results of this study will clarify the role of parkin in genetic susceptibility and foster the development of genetic testing guidelines. The consistent finding that at least 30 percent of PD patients with parkin mutations are heterozygotes, despite that fact that inheritance was initially described as recessive, and new availability of commercial testing make this study both critical and timely. -

Principal Investigator: NELSON, LORENE M
Grant Number: 5R01NS031964-08
Title: Environmental and Genetic Risks for Parkinson's Disease

Abstract: The primary goal of this project is to investigate whether certain environmental exposures and genetic variants, either alone or in combination, affect the risk of developing Parkinson's disease (PD). We will investigate mechanisms that may explain the consistently observed inverse associations of cigarette smoking and caffeine consumption with PD, and the role of residential pesticide exposure on the risk of PD. In addition, existing and newly-identified polymorphisms in the coding and regulatory regions of candidate genes will be investigated, including genes that code for: (1) endogenous enzymes involved in metabolism of tobacco or caffeine, or in the detoxification of putative toxicants for PD, (2) proteins involved in dopamine regulation or metabolism, and (3) proteins that play a role in protein degradation and aggregation in dopaminergic neurons. We propose to expand a recently completed case-control study in a large health maintenance population of more than 500 newly diagnosed PD cases and 500 controls. Because preliminary data show that the strongest associations of genetic variants were observed among PD cases with early age at diagnosis (age less than or equal to 60), we will identify approximately 330 additional such cases along with age- and sex-matched controls. This new sample will be combined with that of the previous study, resulting in approximately 420 young diagnosis cases and 430 older diagnosis (age greater than 60) cases to be compared with 870 age- and gender-matched controls. Detailed information will be collected from all study subjects using in- person and telephone structured interviews including information on cigarette smoking, caffeine intake, and residential exposure to pesticides, along with other putative risk factors. Venous blood samples will be drawn for DNA extraction and genotyping assays for the gene polymorphisms of interest. By examining genetic polymorphisms within a group of carefully chosen candidate genes, in combination with extensive information about common environmental exposures, we hope to advance knowledge regarding both genetic and modifiable environmental risk factors for Parkinson's disease. -

Principal Investigator: O'MALLEY, KAREN L
Grant Number: 2R01NS039084-05A1
Title: Mechanisms of Neuronal Death in Parkinson's Disease

Abstract: Oxidative stress is a major factor in Parkinson's Disease (PD). Dopamine (DA) itself is easily oxidized to quinone derivatives and reactive oxygen species (ROS) that impair energy metabolism and form adducts with proteins such as upsilon-synuclein. Because pharmacological depletion of DA in animal models is confounded by non-specific peripheral and central nervous system effects, the role of DA oxidation in nigral cell death has been previously impossible to address. Thus a key unanswered hypothesis in this field is that DA oxidation is a major contributor to the death of dopaminergic neurons in PD. The proposed studies address several aspects of this hypothesis including the interaction of known environmental factors in triggering DA oxidation. Specifically, the hypothesis that the DA-releasing potential of the parkinsonism-inducing drug, MPP+, is due to its ability to exchange with DA and/or to reduce intracellular pH gradients will be addressed using newly derived mice expressing enhanced green fluorescent protein from a dopaminergic locus (TH+/eGFP). Primary cultures derived from these animals as well purified synaptosomal and vesicular preparations from dopaminergic terminal fields will be used in combination with fluorescent and radioactive probes to determine the temporal aspects of DA release, intracellular membrane changes, ROS formation, ATP loss, etc in response to toxin treatment. In addition, the hypothesis that DA oxidation contributes to the death of dopaminergic cells will be directly tested in vivo using animals genetically engineered to have different levels of DA production. Behavioral, oxidative and immunocytochemical criteria will be used to establish the role of DA in both the acute and chronic MPTP model of PD. To test whether DA depletion prevents ROS, new methodologies to detect in situ ROS will be used with a battery of antibodies directed against nitrotyrosine, nitrated alpha-synuclein, etc. to temporally evaluate ROS formation following acute or chronic MPTP administration in DA deficient and wild type animals. Taken together, the proposed studies will determine whether DA oxidation plays a central role in the death of DA synthesizing cells and provide insights impossible to obtain from standard animal models. Knowledge of the source and cascade of events surrounding DA-induced free radical formation will help answer risk-benefit controversies surrounding the use of dopamine replacement therapies as well as facilitate the development of new drugs and/or treatment strategies in the pathogenesis of PD. -

Principal Investigator: PALLANCK, LEO J
Grant Number: 1R21NS048362-01
Title: Mutational Analyses of Drosophila DJ-1 Homologs

Abstract: Parkinson's disease is a prevalent neurodegenerative disorder characterized by tremors, rigidity, and bradykinesia. These symptoms arise from the degeneration of dopaminergic neurons in the substantia nigra. The cellular and molecular mechanisms responsible for neurodegeneration in Parkinson's disease remain poorly understood, although genetic and environmental factors both appear to play contributing roles. Recently, loss-of-function mutations in DJ-1, a gene of unknown function, were found to be responsible for an autosomal recessive form of Parkinson's disease. To explore the normal biological function of DJ-1, and the mechanism by which loss of DJ-1 function results in neurodegeneration, we propose to subject a pair of highly conserved Drosophila DJ-1 homologs (designated DJ-1a and DJ-1b) to mutational analysis. DJ-1a and DJ-1b function will be perturbed using P element mutagenesis, gene-targeting and double stranded RNA interference methods. The phenotypes resulting from perturbation of these genes will be fully characterized, including an analysis of dopaminergic neuron integrity. Additionally, we will characterize the global gene expression changes resulting from loss of DJ-1a and DJ-1b function and initiate screens for genetic modifiers of the DJ-1a and DJ-1b phenotypes to elucidate the biochemical pathways in which these genes function. This work should clarify the normal cellular role of DJ-1 and provide a foundation for further hypothesis-driven investigation of DJ-1 function. -

Principal Investigator: RACETTE, BRAD A
Grant Number: 5K23NS043351-03
Title: GENETICS OF PARKINSON DISEASE IN THE AMISH

Abstract: The applicant is a neurologist and movement disorders specialist with three years of post-fellowship, faculty experience involving clinical care, clinical trials, and clinical research into etiologic risk factors for PD including genetic factors. The goal of this career development award is to provide the applicant with comprehensive training in genetic epidemiology through course work, individual tutorials, and practical application of gene mapping techniques to a multi-incident Amish family with Parkinson Disease (PD). PD is a neurodegenerative disorder that produces substantial disability for nearly 1 million people in North America. There is no known cause of the disease in the majority of patients; however, a genetic etiology has been found in a few rare multi-incidence families. Identification of such genes and subsequent determination of the cell biological effects of these mutations will provide important clues to the pathophysiology. Each new mutation discovered adds critical converging evidence about pathophysiological mechanisms common to all to those affected with PD. We have identified 27 members of a large Amish family with clinically typical PD and have excluded known PD genetic mutations. However, we still need to prove that PD is inherited in this pedigree. We will use two different methods to prove that PD in this kindred has a genetic basis. The first approach will assume an autosomal recessive model of inheritance and use genetic marker data provided by CIDR on our subjects to perform homozygosity mapping. A second approach will be to calculate a kinship coefficient to determine if the affected members of the pedigree are "more related" than randomly selected age-matched individuals from the same population. Finally, we will test whether [18]FDOPA PET permits the conversion of some people identified clinically as possible or probable PD in to PET-confirmed PD and thereby functioning as an endophenotype for disease state. This family provides a unique opportunity for the candidate to become a productive independent investigator in genetics of Parkinson Disease and other movements disorders and to develop skills needed for interpretation of [18]FDOPA PET.-

Principal Investigator: RICHARD, IRENE H
Grant Number: 5K23NS002184-05
Title: MOOD FLUCTUATIONS IN PARKINSON'S DISEASE

Abstract: The candidate has a clinical background in neurology with an expertise in movement disorders and has completed a two year NIH-funded fellowship through the Department of Neurology in Experimental Therapeutics. This fellowship provided the candidate with both theoretical knowledge and practical experience pertaining to the design and conduct of clinical trials. She has focussed most of her efforts thus far on the understanding and treatment of the behavioral aspects of Parkinson's disease (PD) The candidate's short term goals include the following: 1) to increase her knowledge of basic pharmacology and gain experience using techniques relevant to pharmacologic mechanism oriented research, 2) to gain a better understanding of molecular medicine, 3) to obtain training in psychiatric assessment techniques, 4) to expand her knowledge of areas fundamental to clinical investigation including biostatistics, epidemiology and outcomes research. The focus of her research plan during this career development award will be understanding mood fluctuations in PD. Mood fluctuations have been reported in up to 2/3 of advanced PD patients who experience motor fluctuations. These can be frequent, dramatic and distressing. Research involving the phenomenology and underlying mechanisms of mood fluctuations in PD has been limited. The specific aims of this study are to: 1) better understand the phenomenology of mood fluctuations in PD (frequency, quality, magnitude), 2) better understand the relationship between mood fluctuations and more pervasive depressive disorders in PD, 3) clarify the temporal relationship between changes in mood and motor states in PD, 4) elucidate the neurobiological mechanisms of changing mood states in PD and to determine, in particular, whether mood fluctuations in PD are the result of dopamine dysregulation, and 5) gather preliminary information regarding the optimal treatment of mood disorders in PD. These findings may lead to the development of therapeutic interventions for patients with PD who suffer from these disabling fluctuations on a daily basis. It may also provide a better understanding of the mechanisms responsible for more pervasive forms of depression in PD, and perhaps even in primary psychiatric mood disturbances. -

Principal Investigator: ROCCA, WALTER A.
Grant Number: 5R01NS033978-09
Title: EPIDEMIOLOGY AND GENETICS OF PARKINSON'S DISEASE

Abstract: Parkinson's disease (PD) is a common and disabling condition in the expanding elderly population of the US and worldwide. Its etiology remains unknown and both genetic and environmental factors have been suspected. The long-term goal of the proposed studies based in different sampling is to clarify the etiology of PD and to identify means to prevent it. Three independent but related studies based on different sampling and measurement strategies are proposed. The hypotheses tested derive directly from our current work and preliminary findings. A case control study will include 800 cases of PD referred to the Mayo clinic from a 120 mile radius or from a 5 state region and 800 controls free pf PD and parkinsonism matched by age (+ 2 years), sex and region of residence. Controls from the general population will be identified from health care financial administration lists for cases aged 65 years or above and through random digit dialing for cases below 65 years. Exposures will be accessed through direct telephone interview, and will include tobacco, coffee, and alcohol use; markers of novelty seeking behavior; and, for women, use of estrogen replacement therapy after menopause and other reproductive and estrogen related factors. A first historical cohort study will test the association between unilateral and bilateral oophorectomy before menopause and PD in an established population based cohort. The study will include 2,533 women who underwent oophorectomy in 1950-1987 while residing in Olmsted County, MN and 2,533 women of the same age and residence who did not undergo oophorectomy. A second historical study will test the association between personality traits measured by the Minnesota Multiphasic Personality Inventory (MMPI) and PD in an established research cohort. This study will include 8,775 persons who underwent MMPI testing in 1962-1965 while residing in Minnesota. The proposed case-control study is strong because it has adequate statistical power to confirm our preliminary findings on the role of estrogen in PD and to explore the link between substance use and novelty seeking behaviors in PD. All interviews with case and controls will be direct, the proposed oophorectomy cohort study is strong because of its cohort design, its population-based sampling, its adequate statistical power, and because of the expected high rate of follow-up through both passive and active strategies. The proposed MMPI cohort study is strong because of its cohort design, its adequate statistical power and because of our extensive experience with tracing and interviewing individuals. These three studies will contribute greatly to understanding the causes and possible prevention of PD by exploring novel hypotheses and by

Principal Investigator: RON, DAVID
Grant Number: 3R21NS043628-02S1
Title: Endoplasmic Reticulum Stress and Parkinson's Disease

Abstract: Unavailable

Principal Investigator: ROSS, GEORGE WEBSTER
Grant Number: 2R01NS041265-05
Title: Risk Factors for Pathologic Markers of Parkinson Disease

Abstract: The purpose of this continuation application is to further study two neuropathologic markers of Parkinson's disease (PD), neuronal loss in the substantia niqra (SN) and diminished striatal dopamine levels, in brains of Japanese-American male decedents who were participants in the population based Honolulu Heart Program/Honolulu-Asia Aging Study. These are used as continuous endpoints to identify risk factors utilizing exposure data accumulated prospectively over the past 39 years. Findings include significantly lower SN neuron densities in PD cases compared to controls without PD. Further, duration of PD is highly correlated with SN neuron density. Brains with incidental Lewy bodies have intermediate mean densities. These relationships are strongest for the ventrolateral quadrant. Mid-life risk factors found preliminarily to predict low SN neuron density at death include high total kilocalorie intake, dietary iron and manganese, non-smoking of cigarettes, work on a sugar or pineapple plantation, high body mass index, increased time spent in edentary activity, and (in late life) slowed reaction time. Contrary to expectation, an association of advanced age with deceased SN neuron density was not found. To assess the influence of age with qreater certainty a new Aim is proposed: measurement of SN neuron densities in study subjects dying at a younger age, using archived materials from 160 cohort autopsies done prior to 1991. A second important and unexpected finding is of remarkably low SN neuron densities in the absence of Lewy bodies, but in association with parkinsonian signs, in a subset of the decedents. A second new Aim is proposed to extend investigations of this subset by applying a-synuclein immunohistochemistry to areas of brainstem, limbic regions, cortex, and olfactory bulb. These histopathologic studies will help to determine if the subset of decedents with isolated SN neuron loss represents a prodromal phase of PD, or a pathogenesis not associated with a-synucleinopathy. Continuation will allow accrual of SN neuron density measurements for 800 total autopsies, and 440 striatal dopamine assays. The greater numbers will dramatically enhance statistical power for substantiating risk factors preliminarily identified or suspected. This will also provide opportunity to examine the influence of a wider age range, as well as additional occupational, dietary, medical, constitutional, and environmental exposures on SN neuron density and striatal dopamine levels. -

Principal Investigator: SARANG, SATINDER S
Grant Number: 1R43NS050920-01
Title: PESTICIDE-SYNUCLEIN INTERACTIONS AS RISK FACTORS FOR PD

Abstract: Parkinson's disease (PD) and other age-associated neurological disorders represent one of the largest unmet medical needs in developed countries. However, the discovery of improved diagnostics and therapeutics for these disorders is hampered by incomplete understanding of underlying disease mechanisms and risk factors. Oxidative stress, mitochondrial dysfunction, and protein aggregation have been implicated as major mechanisms causing dopaminergic neuronal loss in PD. Epidemiological studies have revealed an association between pesticide exposure and PD, and pesticides that cause oxidative stress and mitochondrial dysfunction, such as rotenone and paraquat, are used in cellular and animal models of PD. Furthermore, interactions between pesticides and the PD-linked gene alpha-synuclein have been postulated. Although almost 1000 pesticide active ingredients are currently marketed, these compounds have not been systematically screened for neurotoxicity in cellular or animal models of PD. The identification of pesticides that interact with alpha-synuclein to cause neurodegeneration may lead to the discovery of novel candidate risk factors and more representative disease models for PD. For this proposal, investigators at Cambria Biosciences will exploit a published moderate-to-high throughput neuronal cell-based model of PD, with the goal of identifying individual pesticides and synergistic pesticide combinations potentially involved in the pathogenesis of PD. Our established cellbased model of PD will be used to screen -approximately 350 registered pesticides to identify neurotoxic pesticides. Our specific aims include: (1a) identifying neurally-active pesticides that induce cell injury to two PD-like cell lines that stably express wild type (WT) human alpha-synuclein and mutant A53T alpha-synuclein; (1b) identifying any synergistic effects of neurotoxic pesticides in inducing cell damage in these a-synuclein-expressing neuronal cells; and (2) characterizing the activity of these neurotoxic pesticides and pesticide combinations using primary mature mesencephalic DA neurons. The identified neurotoxic pesticides will be employed in follow-on Phase II studies for the development of improved in vitro and in vivo PD models, which will ultimately be used to screen for neuroprotective compounds as part of a comprehensive drug discovery program. -

Principal Investigator: SAUNDERS-PULLMAN,
Grant Number: 1K23NS047256-01
Title: Gender and Hormonal Differences in Parkinson's Disease

Abstract: This application is directed to the career development of Dr. Rachel Saunders-Pullman as a clinical researcher in the study of hormonal effects on Parkinson's Disease and other neurodegenerative disorders. During the period of this grant she will focus on gender differences and the role of hormones in the development of Parkinson's disease (PD). As hormone physiology is complex, and estrogenic compounds can be carcinogenic as well as beneficial, the assessment of the role of estrogen and gender differences and the planning of clinical trials requires multi-disciplinary knowledge and training. The candidate has constructed a training program with areas of emphasis in epidemiology, neuroscience and neuroendocrinology, cognition and movement disorders using an integrated plan of research, coursework, lectures, and rounds. Her primary sponsor, Dr. Richard Lipton will oversee the methods and the candidate's development in epidemiology and cognition as well as the overall training. The co-sponsor, Dr. Susan Bressman will provide guidance in the field of movement disorders. A consultant team of Dr. Anne Etgen, Dr. Nanette Santoro and Dr. Charles Hall will complement with expertise in neuroscience and neuroendocrinology and biostatistics, respectively. In order to address gender related differences in risk of PD and clinical features of PD, and whether hormonal factors account for these differences, three separate studies with differing study designs are proposed. These studies were designed to provide complementary answers to specific research questions and to give the candidate hands-on, mentored exposure to three major types of epidemiologic research 1) a clinic-based prospective study of gender differences in the natural history and disease course in early PD, 2) a case-control study of pharmacy records to assess whether exogenous estrogen decreases the risk of PD and 3) a cross-sectional and a prospective evaluation of the role of gender and endogenous hormone levels on motor control measures in aging and pre-clinical parkinsonism in an established cohort study. Through the aggregate of the training program and supervised research, the candidate will emerge as an independent researcher well prepared to answer questions of the role of hormones in neurodegenerative diseases and movement disorders. -

Principal Investigator: SILVERMAN, RICHARD B
Grant Number: 1R01NS047331-01A1
Title: Celestrols for Treatment of Neurodegenerative Diseases

Abstract: The expression of molecular chaperones has been shown to suppress protein misfolding/aggregation and cellular toxicity phenotypes in model systems associated with Huntington's Disease, Alzheimer's Disease, Parkinson's Disease, and ALS. A feature common to diseases of protein conformation is the appearance of folded intermediates that self-associate to form protein aggregates and inclusions. The molecular chaperones Hsp90 and Hsp70 sequester damaged proteins that appear in cells exposed to physiological and environmental stress. The ability of molecular chaperones to suppress the cellular toxicities associated with expression of these "toxic" proteins may be due to the intrinsic properties of chaperones to capture and suppress the appearance of folded intermediates. Therefore, we propose that the identification of small molecules that elevate the expression of genes encoding heat shock proteins and molecular chaperones should lead to the development of novel therapies beneficial to the prevention of neurodegenerative diseases. The rationale for this proposal is based on results obtained by our laboratory and others who participated recently in a screening program organized by the NINDS, Huntington Disease Society of America, Hereditary Disease Foundation, and the ALSA to identify new drugs for treating these diseases. A search was carried out for drugs that activate the heat shock response; the most effective compound identified was the natural product celastrol. Synthetic analogs of celastrol will be prepared to optimize its effectiveness as a regulator of the heat shock response and a suppressor of neurotoxicity and to determine its mechanism of action as an activator of the heat shock response. To probe the function of celastrol as a potential therapy for neurodegenerative diseases, the following Specific Aims will be addressed: (1) Synthesize analogs of celastrol that induce the human heat shock response using a heat shock promoter-reporter assay in human tissue culture cells. (2) Determine the mechanism of action of celastrol (or an analog). The working model is that celastrol activates the heat shock response by inducing heat shock transcription factor HSF1. The mechanism by which HSF1 activity is induced by celastrol will be determined. It also will be determined whether celastrol, by virtue of its ability to activate the expression of chaperones, can reduce the aggregation and neurotoxicity of the Huntington Q64 protein expressed in a human SH-SY5Y neuroblastoma cell line. (3) Studies will be carried out to identify the binding target for celastrol using molecular biological and biochemical techniques. Identified target(s) will then be cloned and characterized. Results of these studies

Principal Investigator: SMEYNE, RICHARD J
Grant Number: 1R21NS045906-01A2
Title: Role of Environment in Neuroprotection

Abstract: PD is a debilitating neurological disorder that strikes 20 per 100,000 persons greater than 50 years of age. The cause of >90% of all PD cases are unknown. However, the discovery of the meperidine by-product 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has provided a useful model of Parkinsonism that appears to recapitulate the pathology of the disease seen in man. Exposure to this prototypical "environmental toxin" causes a selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). MPTP is a lipophillic molecule that rapidly enters the brain and is metabolized to MPP+ through a series of intermediates to MPP+ by the enzyme MAO-B. MPP + is a substrate for dopamine uptake mechanisms and it accumulates intraneuronally and interferes with complex I of the electron transport chain. We have recently shown that the glial cell is the critical cell for conferring protection or susceptibility to this toxin. Since PD is progressive, both in terms of cell loss and symptomotology, it would be of tremendous clinical value if there were cell biological, pharmacological or non-pharmacological methods that could attenuate cell loss; with or without interruption of the disease triggers. Alternatively, at the least, it would be important to slow the progression of cell loss once symptoms arose. There is a significant literature, dating back to the late 1700's that altering an animals' environment can lead to neurological changes. These changes are manifested as increased brain size, increased learning, and recently it has been shown that environment can increase neurogenesis. Recently, we have preliminary data to suggest that mice raised in an "Enriched Environment" (EE) are protected from MPTP toxicity. In this application, we will study and further establish the EE model. In addition, we will examine if the components (exercise, alterations in environmental complexity and/or social interactions) of the EE can confer neuroprotection. In addition, we will examine the role of the neurotrophin BDNF in EE-dependent neuroprotection. The work proposed and subsequent results generated in the application will be used as pilot data. We believe that the EE model may provide a new approach to prevention of PD symptomatology as well as other neurodegenerative disorders. -

Principal Investigator: SMEYNE, RICHARD J
Grant Number: 2R01NS039006-04A2
Title: Genetics of MPTP-Induced Parkinsonism

Abstract: Parkinson's disease (PD) is a debilitating neurological disorder that strikes 20 per 100,000 persons greater than 50 years of age. It is estimated that 1 million US citizens have PD, with adults over 60 having a 1 in 20 chance of getting PD. At an average per capita cost of $6000.00 year/patient, the total cost of the disease approximates $6 billion dollars, of which 85% is borne to private and government insurance agencies. Since the population of the world is getting progressively older, the number of people suffering from this disease should substantially increase within the next several decades. The cause of >90% of all PD cases is unknown. Current hypotheses on the etiology of idiopathic PD (IPD) state that there is an interaction of some as yet unknown environmental agent with a genetic predisposition to its effects. The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has provided a useful model of Parkinsonism that appears to recapitulate the pathology of the disease seen in man. Exposure to this prototypical "environmental toxin" causes a selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). In mice, the effects of MPTP are strain dependent. We have used a QTL analysis to demonstrate that the gene underlying strain differences is located on chromosome 1. Within this chromosomal region, one gene: glutathione-S-transferase pi2 functions within the detoxification pathway for exogenous agents. In this application, we propose to study the structure and function of this gene and its related family members. Four specific aims are proposed: 1) Determine if there are any differences in the sequence and expression of GSTp2 and related family members in MPTP-resistant and sensitive strains of mice. 2) Examine the effects of blockade or transfer of GSTpi on cell death following administration of MPTP in vitro and in vivo; 3) Develop the rotenone model of experimental Parkinsonism in mice and determine if GSTp2 is altered in response to rotenone; 4) Determine if there are structural or expression differences in GSTpi levels in humans with Parkinson's disease. The results of this study should lead to a better understanding of the pathogenesis of experimental and possible human Parkinson's disease. This identification of GSTp2 as a candidate gene could also lead to the identification of diagnostic measures and point to potential therapies for early intervention in this devastating illness. -

Principal Investigator: Tanner, Caroline M.
Grant Number: 5R01NS040467-05
Title: TWINS AND RISK OF PD: A CLINICAL AND IMAGING STUDY

Abstract: The long-term goal of this research is to determine the relative contributions of genetic and environmental factors in the etiology of typical Parkinson's disease (PD) by comparing concordance rates in MZ and DZ twin pairs, at least one of whom has Parkinson's disease. This proposal extends our recent results in an irreplaceable cohort, the NAS/NRC World War II Veteran Twins cohort, which showed nearly identical concordance rates in monozygotic and dizygotic twin pairs with typical late onset (over age 50) Parkinson's Disease. These results strongly implicate environmental factors in the pathogenesis of Parkinson's disease, since one would expect monozygotic twins to show a much higher concordance rate than dizygotic twins if Parkinson's Disease were genetically determined. However, without follow-up we cannot be certain that unaffected cotwins would not have eventually developed the disease, thus changing the study outcome. We propose to assess the presence of both clinical parkinsonism and abnormal striatal dopamine function in twin pairs discordant for Parkinson's disease, and to compare concordance rates by zygosity. Aim 1 will determine if long-term follow-up will change Parkinson's disease concordance ratios in monozygotic and dizygotic twins. We expect to add at least 100 newly diagnosed twin pairs and to re-assess diagnosis in 140 prevalent discordant pairs. The second aim will be to compare the concordance rates in monozygotic twins and dizygotic twins, for either Parkinson's disease or abnormal striatal dopamine function as measured using dopamine transporter imaging with [123I]beta-CIT (2beta-carbomethoxy-3beta-(4-iodophenyl) and SPECT. In the third aim, we will compare concordance rates by zygosity for an abnormal rate of decline in striatal dopamine function. The mean annual decline in striatal dopamine uptake will be estimated by using [123I]beta-CIT uptake separated by, on average, two years. These studies, by virtue of utilizing both clinical and imaging measures, should determine clearly and beyond a doubt if the earlier twins study was flawed by virtue of missing pre-clinical cases of Parkinson's disease. This in turn could help set the research agenda on the cause of Parkinson's disease for years to come. -

Principal Investigator: TESTA, CLAUDIA M
Grant Number: 5K08NS044267-03
Title: Mitochondrial dysfunction in neurodegenerative disease

Abstract: Like most neurodegenerative disorders, Parkinson disease (PD) has a chronic, slowly progressive course, selective neuronal loss, and a small percentage of familial cases caused by mutations in widely expressed genes. A simplified, reproducible and relevant model system that allows study of progressive neuronal injury would permit us to examine mechanisms of chronic neurodegeneration in PD, and allow us to screen potential neuroprotective agents. Organotypic "slice" culture models offer major advantages in that they are simplified compared to in vivo models, yet unlike dissociated cell cultures they involve the use of mature neurons, remain viable in culture for months, and maintain substantial intact circuitry and neuronal-glial interactions. We propose to characterize and use such a model to specifically examine mechanisms of neuronal injury in PD. Mitochondrial dysfunction has been proposed as a factor underlying dopaminergic cell loss in PD. There is growing evidence of decreased mitochondrial function and increased oxidative stress in human PD. In a new animal model of PD, systemic infusion of the mitochondrial toxin rotenone, an organic pesticide, causes degeneration of the nigrostriatal pathway that is highly selective, even in the presence of global mitochondrial inhibition. In the current proposal we will: 1) Optimize and characterize a rotenone model of PD in chronic organotypic slice cultures. We present data from preliminary studies demonstrating the successful use of slices containing substantia nigra pars compacta dopaminergic neurons for this purpose. 2) Exploit the unique advantages of this system to investigate the mechanisms of action of mitochondrial inhibition. We will examine the role dopamine itself plays in neuronal vulnerability, and look for evidence of oxidative damage and apoptotic cell death. 3) Investigate the interaction of genetic defects with environmental stressors in PD. We will use transgenic mouse models to examine how rotenone interacts with genetic mutations that produce familial PD. We will study how underlying genetic lesions that affect oxidative stress and apoptosis pathways may predispose cells to damage from exogenous toxins. 4) Test potential neuroprotective agents in a model of chronic neurodegeneration that is highly relevant to PD. The research outlined above is part of a customized five-year plan of training and career development for the Principal Investigator. The proposal includes active mentoring by experienced scientists, access to diverse resources, and an environment uniquely suited to help the PI develop as an independent physician scientist.-

Principal Investigator: VANCE, JEFFREY M
Grant Number: 2P50NS039764-06
Title: The Genetics of Parkinsonism

Abstract: This is a continuation application of our very successful Morris K. Udall Parkinson Disease Research Center of Excellence, seeking to identify genes that contribute to risk of developing PD. Four projects and two cores are proposed. Project I, "Candidate genes and complex interactions in PD," continues the association studies of potential susceptibility genes with PD, derived from biological candidates and the gene expression studies of Project II. Additional specific aims are gene-gene and environmental-gene interactions. Project II, "Expression Analysis and Genomic Convergence," continues and extends our expression studies of tissue obtained by our autopsy program by adding examination of the putamen and the anterior olfactory nucleus to the SN, as well as using Laser Capture Microscope to investigate specific cell types. Genes identified in project II will be tested for association in collaboration with Project I. Project III, "Mitochondrial genetics and PD," builds upon our finding of a highly significant association of mitochondrial-encoded proteins with PD, specifically the haplogroups J and K and SNP 10398, which lies in the complex I subunit ND3. Using cybrids, it looks for functional differences associated with these different mitochondrial haplogroups. It also will examine nuclear mitochondrial genes with significant differential expression in Project II for association with PD. Project IV, "Association Mapping in PD Linkage Regions," will identify PD genes in regions of linkage on chromosomes 5, 8, and 9 through a new approach, genomic "iterative" association mapping, using a new DNA pooling strategy. Once the strongest region of association is identified, haplotype-tagging will be utilized to fine map the region further. Genes lying in the region will be tested for association with PD. The projects depend heavily on our productive cores. In Core B we continue our very successful collection of PD patients and siblings, as well as our prospective autopsy program. Core C provides neuropathology support for investigation and diagnoses of autopsy material, brain banking and genotyping support for the projects. We believe that by utilizing these different but integrated approaches and resources we will be able to define the genetic contributions to PD. -

Principal Investigator: WOOTEN, GEORGE F
Grant Number: 3P50NS039788-05S1
Title: MITOCHONDRIAL ETIOLOGIES OF PARKINSON'S DISEASE

Abstract: Unavailable